

WHAT IS CLAIMED IS:

- Sub A1
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1. A method for creating a target single-stranded region in double-stranded DNA comprising:
- (a) nicking at least one site bordering the target region in double-stranded DNA with at least one site-specific nicking endonuclease; and
- (b) subjecting the nicked DNA to conditions where the target region is selectively denatured.
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2. The method of claim 1 wherein the target single-stranded region comprises a gap in the double-stranded DNA and wherein the method comprises:
- (a) nicking at least two sites bordering the target region in one strand of double-stranded DNA with at least one site-specific nicking endonuclease; and
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- (b) subjecting the nicked DNA to conditions where the target region is selectively denatured.
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3. The method of claim 1 wherein the target single-stranded region comprises at least one terminus in the double-stranded DNA and wherein said method comprises:
- (a) nicking at least one site bordering the target region in a first strand of the double-stranded DNA with at least one site-specific nicking endonuclease, wherein the
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- second strand of the double-stranded DNA has at least one break bordering the target region; and

(b) subjecting the resulting DNA to conditions where the target region is selectively denatured.

4. The method of claim 3 wherein the break in the second strand is pre-existing.

5. The method of claim 3 wherein the break in the second strand is produced by a site-specific endonuclease.

6. A method of joining nucleic acid molecules comprising creating a target single-stranded DNA region in a first molecule in accordance with the method of claim 1 and adding a second molecule containing a nucleic acid single-stranded region complementary to that contained on the first molecule.

7. A method of joining nucleic acid molecules comprising creating a first DNA molecule with a target single-stranded DNA region of at least three nucleotides in accordance with the method of claim 1 and adding a second molecule containing a nucleic acid single-stranded region complementary to the target single-stranded region at the first molecule.

8. A method of joining nucleic acid molecules comprising creating a first DNA molecule with a target single-stranded DNA region of at least nine nucleotides in accordance with the method of claim 1 and adding a second

molecule containing a nucleic acid single-stranded region complementary to the target single-stranded region of the first molecule.

5 9. A method of joining nucleic acid molecules comprising creating a first DNA molecule with a target single-stranded DNA region of at least 12 nucleotides in accordance with the method of claim 1 and adding a second molecule containing a nucleic acid single-stranded region
10 complementary to the target single-stranded region of the first molecule.

10 10. A method of joining nucleic acid molecules comprising creating a first DNA molecule with a target single-stranded DNA region of at least 18 nucleotides in accordance
15 with the method of claim 1 and adding a second molecule containing a nucleic acid single-stranded region complementary to the target single-stranded region of the first molecule.

20 11. A method of attaching a molecular probe to DNA comprising annealing a complementary single-stranded nucleic acid region on the probe to a single-stranded DNA region produced by the method of claim 1.

25 12. A method for the purification of a specific DNA fragment comprising annealing a single-stranded DNA region

produced by the method of claim 1 to a complementary single-stranded nucleic acid attached to a solid support.

5 13. A method for producing a branched nucleic acid molecule comprising:

- (a) producing a single-stranded gap in double-stranded DNA according to the method of claim 2; and
- (b) annealing to the single-stranded region of step (a) a second nucleic acid molecule containing a single-stranded region complementary thereto.
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14. A nucleic acid molecule comprising of at least two fragments joined by single-stranded termini produced by the method of claim 3.

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15. A nucleic acid molecule which comprises three or more fragments joined by single-stranded termini produced by the method of claim 3.

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16. A nucleic acid molecule which comprises at least two fragments joined by single-stranded termini, in which at least one terminus is produced by the method of claim 3.

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17. A circular nucleic acid molecule produced by joining at least two fragments containing at least one terminus produced by the method of claim 3.

18. A DNA vector produced by joining fragments containing at least one terminus produced by the method of claim 3.

5 19. A method for assembling a vector with multiple, interchangeable parts comprising element sets containing:

- (a) a replication origin and associated control sequences; and
- (b) a selectable marker; and
- 10 (c) one or more of the following element sets:
 - (i) promoters and associated control elements;
 - (ii) coding sequences allowing production of fusion proteins;
 - (iii) transcription terminators;
 - 15 (iv) regulatory proteins; and
 - (v) gene coding regions;

wherein each element set has unique common cohesive termini, allowing joining of elements within an ordered assembly, and interchange of elements in each set in the
20 assembly.

20. A method for generating a DNA fragment with specific single-stranded termini comprising (a) inserting a target DNA fragment between two sets of site-specific nicking
25 sites, wherein said sites are situated to generate single-stranded termini in accordance with the method of claim 3, and (b) digesting the resulting construct with the cognate site-specific nicking endonuclease or endonucleases to

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release the target DNA fragment containing the single-stranded termini.

21. A method for generating a DNA fragment with
specific single-stranded termini comprising (a) inserting a
target DNA fragment between two sets of N.BstNBI sites,
wherein such sites are situated to generate single-stranded
termini in accordance with the method of claim 3, and (b)
digesting the resulting construct with N.BstNBI endonuclease
to release the target DNA fragment containing the added
single-stranded termini.

Add A2

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B1